Limonene, a Citrus Extract, for Control of Mealybugs and Scale Insects

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ABSTRACT In a series of bioassays with mealybugs, aqueous solutions of 1% limonene were tested that used from 0.50 to 1.50% all purpose spray adjuvant (APSA)-80 as an emulsifier/surfactant. The two ingredients were added to water or to 0.1% Silwet L-77, an agricultural surfactant. Using 1% limonene, 0.75% APSA-80 and 0.1% Silwet L-77, a semitransparent mixture (primarily a microemulsion) was obtained that was safe for most plants and provided good control of mealybugs when sprayed or used in 1-min dips. Used at half strength, this mixture controlled ≥99% of whiteflies, whereas the full-strength mixture controlled from 69 to 100% of mealybugs and scales, including ≥93% control of root mealybugs. In side-by-side greenhouse tests, this mixture was superior to a 2% solution of insecticidal soap or a 2% solution of horticultural spray oil. Mortality of green scales on potted gardenia plants averaged 95, 89, and 88% on plants sprayed with limonene, insecticidal soap, or horticultural oil, respectively. In a related test, these same sprays killed 44.1, 22.7, or 12.5% of third and fourth instar clustering mealybugs, respectively. Limonene has promise as a safe, natural pesticide for insect pests on tolerant plants. Although 1% limonene solutions damaged certain species of ferns, gingers and delicate flowers, they caused no damage to ornamentals with thick, waxy leaves, such as palms, cycads, and orchids.

KEY WORDS monoterpenes, mealybugs, scales, surfactants, natural insecticides

Mealybugs and scales are serious pests of agriculture and ornamental gardens, reducing the vigor of perennial crops by removing plant sap, secreting toxic enzymes, or transmitting plant diseases (Khoo 1974). In addition, these insects are important quarantine pests that impede international trade of fruits, vegetables, and ornamental plants. They are notoriously difficult to control with conventional insecticides (Schread 1970, Hamlen 1977). Mealybugs and scales are protected from sprays by their sedentary habits (making them less likely to contact pesticides), sheltered feeding locations (under leaves, at plant nodes, or on roots within the soil) and the water-repellent waxes that cover their bodies (Donahue and Brewer 1998).

Mineral oil emulsions or solutions of insecticidal soap (potassium salts of fatty acids) are commonly recommended at 1–2% active ingredient, alone or in combination with other insecticides, for control of scale insects and mealybugs on actively growing plants (Tomkins et al. 1996, Donahue and Brewer 1998). The oil or soap helps the mixture "wet" (penetrate) the waxy exterior of these insects.

Our initial goal was to discover synergistic combinations of soaps, oils, and surfactants and other chemicals of low mammalian toxicity that could penetrate the wax of mealybugs and kill them. When limonene was used in our dip tests, it soaked and killed essentially 100% of mealybugs. However, close examination revealed that some of the limonene had collected on the surface of the treatment solution, creating an oily phytotoxic "slick." Several chemicals sold specifically as limonene emulsifiers eliminated the surface slick but yielded emulsions that were not effective in killing target pests. After testing insecticidal soap and a number of commercially available surfactants for their ability to emulsify limonene, we discovered that all purpose spray adjuvant (APSA)-80 (a nonionic sticker/spreader comprised of alkyl aryl alkoxylate + tall oil fatty acids) (Amway Phillippines, LLC, Tambo, Parañaque City, Phillippines) produced the best results without causing damage to the gardenia leaves being used to measure phytotoxicity. We also discovered that adding a small amount of Silwet L-77 (polyalkylene-oxide modified heptamethyltrisiloxane, Loveland Ind., Cambridge, United Kingdom) further reduced surface tension of treatment solutions, leading to smaller air bubbles around the bodies of dipped mealybugs and better wetting of the wax covering their bodies.

The current study addressed three objectives: 1) determine the effects of mixing method and the amount of surfactants used on efficacy against mealybugs and phytotoxicity to gardenia leaves, 2) determine how the amount of surfactant used affected clarity of solutions/emulsions and the size distribution

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of emulsified limonene droplets, and 3) measure efficacy and phytotoxicity of the "best" mixture by using a range of pest and plant species.

Materials and Methods

Technical grade d-limonene (Florida Chemical Company, Inc., Winter Haven, FL) was used in all studies.

Mealybug Bioassays with Different Treatment Solutions. One-minute dip bioassays were carried out to determine which treatment solutions/emulsions containing 1% limonene and various amounts of surfactants provided best control with minimal phytotoxicity. Bioassays used 150-ml beakers, each holding 100 ml of a different treatment solution: 1) limonene added to APSA-80 solution; 2) limonene and APSA-80, first mixed as concentrates before being added to water; 3) limonene and APSA-80, mixed as concentrates and then added to a solution of 0.1% Silwet L-77; 4) limonene and 0.1 ml of Silwet, mixed as concentrates, and then added to a solution of APSA-80; 5) APSA-80 and limonene added sequentially to Silwet solution; 6) distilled water only; 7) APSA-80 alone; and 8) APSA-80 added to a 0.1% Silwet solution. In each bioassay, the rate of APSA-80 was 0.50, 0.75, 1.00, 1.25 or 1.50 ml/100 ml of finished solution. Silwet was used at a rate of 0.1 ml/100 ml. A complete test consisted of five bioassays in which all levels of APSA-80 had been tested; three replications of tests were carried out over time. Solutions were mixed vigorously with a stir bar ($\approx$550 rpm) for $\geq$15 m before use. For mixtures requiring a two-step mixing process (e.g., mixture 4), component mixtures were stirred separately for $\approx$15 m before being combined.

After mixing, solutions were allowed to stand for 1 min before a sample was collected from the surface by using a micropipette to determine whether a surface slick of limonene was present. Limonene in the pipette refracted light differently from the other part of the sample in the pipette. The appearance of each solution was recorded (clear, semitransparent, or milky). Phytotoxicity was measured using gardenia, *Gardenia augusta* (L.) Merr., 'Mystery', branch tips (15–20 cm in length) dipped into beakers holding treatment solutions (or water) for 1 min (one phytotoxicity sample per mixture). After 24 h, phytotoxicity was determined by placing the treated leaves against a light source and looking for damage, which was not always apparent on the surface of the leaf. Limonene damage was manifested as dark areas under the leaf cuticle, or as dark brown or black necrotic spots ($\approx$2–3 mm in diameter) present on the surface of the leaf. Phytotoxicity was rated on a scale of 0 to 3 with 0 being no damage, 1 being slight damage (a few small black spots per 5–10 leaves, but covering <20% of leaf area), 2 being moderate damage (dark spots covering at least 20% of leaf surface, but <50% of leaf surface), and 3 being for heavy damage (deep necrotic pits covering >50% of leaf surface).

Immediately after being used for gardenia dips, treatment solutions were used in bioassays with longtail mealybugs, *Pseudococcus longispinus* (Targioni-Tozzetti), originally collected from gardenia and reared for 3 yr in the laboratory on green beans within ventilated containers. An infested bean piece (5–8 cm in length, one per treatment solution) was submerged for 60 s and then allowed to drip for 5 s before being placed within a 1-liter silk screen-ventilated plastic container that was placed on a shelf in the laboratory. After 24 $\pm$ 2 h at ambient temperature ($\approx$22°C) and photoperiod (no supplemental lighting), insects were examined under a dissecting scope and counted as dead or alive. While counting, each insect was assigned to one of four instars based on a subjective estimate of relative size.

Size of Emulsified Limonene Droplets. To determine the effect of surfactants and mixing method on the quantity and size distributions of limonene droplets, solutions were made using mix methods 1, 2, and 3 containing 0.5–1.5% APSA-80, as described above. From each test solution, four aliquots were examined using an improved Neubauer hemacytometer (Hausser Scientific Company, Horsham, PA) and a phase microscope. From each aliquot, four randomly chosen areas totaling 0.16 mm^2 (0.016-mm^3 volume) were examined. Within each area, droplets and/or particles were counted within four size categories ($\leq$0.005, 0.005–0.01, 0.01–0.025, and $\geq$0.025 mm) based on an estimate of diameter relative to the 0.05-mm length of one of the improved Neubauer squares. For statistical analysis, each aliquot was considered to be an independent observation.

Efficacy and Phytotoxicity of Best Limonene Mixture. Based on 1-min dip bioassay results by using longtail mealybugs and gardenia, mix 3 containing 1% limonene, 0.75% APSA-80, and 0.1% Silwet L-77 was selected as the best mixture, demonstrating good efficacy with low phytotoxicity. This mixture was used in a series of dip and spray tests against various insect species to better determine its potential as an insecticide. Both dip and spray methods were used, because dips, which are generally more effective, might be used for harvested commodities such as fruits, vegetables or cut flowers, whereas sprays would be more practical for potted plants. Insect species easily controlled with the full-strength limonene mixture (such as whiteflies) were treated with solutions containing 0.25 or 0.50% limonene (achieved by the dilution of the full-strength mixture with water). Green scale, *Coccus viridis* (Green); root mealybugs, *Rhizoecus* spp.; and a whitefly species *Aleurothrixus antidesmae* Takahashi were treated on potted gardenia plants in the greenhouse, and hemispherical scale, *Saissetia coffeae* (Walker) was treated on greenhouse grown orchids (*Oncidium* hybrids). Longtail mealybugs were obtained from a laboratory colony on green beans, as previously described. All other insects were collected outdoors on hosts indicated in tables. Infested leaves or potted plants to be treated were divided into experimental blocks based on the degree of infestation, and treatments were randomly assigned within blocks (three to five "blocks" or replications per test). All bioassay tests, whether a spray test or 1-min dip, included a water control. Sprays on excised leaves or leaflets were applied using a hand-sprayer held 10–20 cm from the leaf surface. After treatment, excess treatment solution was allowed to drain away, and the infested material was placed in ventilated plastic containers and kept at ambient temperature in the laboratory (21–25°C with no supplemental lighting) until results were evaluated. Results of spray or dip tests were evaluated from 1 to 7 d after treatment, depending on how much time was required before insects killed by limonene changed color or dried up such that mortality could be accurately assessed. When a significant number of dead insects were present before tests, results were obtained by counting living insects only, and percentage of mortality was calculated based on the reduction of living insects in treatments, as indicated in tables. Tests with green scale used branches 15–20 cm in length placed in individual flasks holding water. After treatment, samples were held in the laboratory for 7 d over a soapy-water moat to prevent cross contamination. Root mealybug tests used ten gardenia bushes in 11.4 liters (3-gal) pots. Pots holding plants were immersed for 1-min in treatment solution or water (five treated plants and five water controls). After 2 d, a knife was used to remove a vertical slice of the outer root ball from each pot and living and dead root mealybugs in each sample were counted for 20 min with the aid of a dissecting microscope.

To facilitate a direct comparison of the efficacy of 1% limonene with 2% insecticidal soap and 2% mineral oil, I used gardenia plants growing in 10-cm pots in the greenhouse infested with green scales and citrus mealybug *Planococcus citri* (Risso). For each insect species, 20 plants were selected and divided into five blocks of four plants each based on the apparent number of insects present. Treatments were assigned at random within blocks as follows: 1) water control; 2) 1% limonene (mix 3 with 0.75% APSA-80 and 0.1% Silwet L-77); 3) 2% Safer Insecticidal Soap (49% potassium salts of fatty acids, Woodstream Corp., Lititz, PA); and 4) 2% SunSpray Ultra-Fine horticultural spray oil (98.8% paraffinic oil, Sun Company, Inc., Philadelphia, PA). Plants infested with green scales were also infested with mealybugs; therefore, efficacy data against mealybugs also was collected from these plants. Plants were thoroughly sprayed with water or treatment solution by using a hand sprayer held at a distance of ≈10 cm from plants and applying uniform force of the spray. Insects were counted on plants 2–6 d posttreatment (6 d was required before scales killed by the treatment changed color) with the aid of a dissecting microscope. Both living and dead mealybugs were counted; for scales, only living insects were counted, because a significant number of scales on plants were dead before treatments due to *Verticillium lecanii* (Zimmermann), a common fungal pathogen.

Phytotoxicity observations were made on a range of tropical cut flowers and foliage in a single test by using 0.5, 1, 2, and 4% solutions of limonene (mix 3, by using a ratio of 1.0, 0.75 and 0.10 of limonene, APSA-80, and Silwet L-77, respectively). Plant material was obtained from a local floral distributor. Four or five stems of each commodity were dipped for 1-min in water or treatment solution, placed in 20-liter buckets partially filled with a 1% solution of Floralife Fresh Flower Food (Floralife, Inc., Walterboro, SC) and held at ambient temperature in the laboratory. Tap water was used as a control for dips. Phytotoxicity data were recorded after 2, 4, and 7 d. Damage observed included presence of water-soaked or necrotic spots, loss of leaf sheen, brown tips on leaves, and wilting. In each damage category, stems were rated on a 0–4 scale, with 0 corresponding to no damage and 4 corresponding to severe damage, all tissues affected. Commodities with an average rating of 1 were considered to have slight damage (damage not readily apparent, commodity still marketable); a 2 rating corresponded to a low level of damage (minor damage but commodity not marketable due to noticeable esthetic damage); 3 corresponded to moderate damage (damage obvious even from several meters distance); and 4 corresponded to high degree of damage. The average damage rating 7 d posttreatment is reported in the data table.

Analysis. Analyses of variance (ANOVAs) and multiple comparison tests (Tukey's honestly significant difference [HSD] test) were carried out using the GLM procedure of SAS Institute software (SAS Institute 2001). Percentage of mortality data were square-root arcsine transformed before analysis (Steel and Torrie 1980, p. 236). To analyze the effect of different mixing methods and different percentages of APSA-80 on the efficacy of 1% limonene solutions against longtail mealybugs, the ANOVA model used block, % APSA-80, mix type, and % APSA-80*mix type as independent, categorical variables. To compare limonene emulsions obtained using mixes 1 and 2 (limonene added to a solution of APSA-80 or to APSA-80, respectively), two ANOVA models were used with the dependent variable being either small droplet size (<0.005 mm in diameter) or large droplet size (≥0.005 mm in diameter) regressed against mix type, % APSA-80, and mix type*% APSA-80 (all variables treated as categorical).

Results

Mealybug Bioassays with Different Treatment Solutions. An average of 277 (SD = 14.2) mealybugs were exposed to each treatment solution (=combination of mix type and given percentage of APSA-80). One percent limonene solution killed from 43.9 to 98.2% of third and fourth instar mealybugs, depending on the percentage of emulsifier (APSA-80) added and the type of mixture used (Table 1). Across APSA-80 concentrations, mixes 3, 4, and 5 killed significantly more mealybugs than mix 2 (Tukey's HSD multiple comparison test, error df = 63, $P = 0.05$); however, there was a significant interaction ($F = 3.1$; df = 4, 63; $P = 0.02$) between APSA-80 concentration and mix method (Table 2). This interaction was largely due to opposite mortality trends associated with mix 1 and

Table 1. Average percentage of mortality of third and fourth instars of longtail mealybugs on beans dipped for 1 min in treatment solutions containing various concentrations of emulsifier APSA-80

% APSA-80	Mix no.[a]	% mortality	SE[b]	Phytotoxicity rating[c]	Appearance of solution[d]
0.50	1	96.1	3.4	1.0	M
	2	43.9	7.1	0.3	M
	3	74.0	3.9	0.3	M
	4	93.4	2.3	0.3	M
	5	92.7	3.8	1.0	M
0.75	1	77.1	13.6	0.0	M
	2	56.4	4.4	0.0	ST
	3	92.1	6.2	0.0	ST
	4	88.1	9.5	0.0	M
	5	84.4	7.5	0.7	M
1.00	1	58.5	22.9	0.0	M
	2	81.1	3.4	0.0	T
	3	73.5	13.3	0.3	T
	4	92.0	3.2	0.3	M
	5	90.4	2.9	0.0	M
1.25	1	75.7	13.6	0.7	ST
	2	69.2	13.2	0.3	ST
	3	91.9	4.5	0.3	ST
	4	89.1	10.4	0.7	ST
	5	74.6	6.1	1.0	M
1.50	1	87.9	4.9	0.3	ST
	2	90.1	2.6	0.3	ST
	3	91.7	5.0	1.3	ST
	4	98.2	1.0	0.0	ST
	5	88.3	5.5	0.7	M

[a] Mix 1, (H_2O + APSA) + Lim; mix 2, (Lim + APSA) + H_2O; mix 3, (Lim + APSA) + (H_2O + Sil); mix 4, (Lim + Sil) + (APSA + H_2O); mix 5 = (H_2O + Sil) + APSA + Lim. Ingredients listed within parentheses were first mixed before being combined with other ingredients. Lim, limonene; Sil, Silwet L-77.

[b] n = 3 replications carried out over time.

[c] Ratings taken on gardenia leaves dipped for one minute (0, no damage; 1, slight damage; 2, moderate damage; 3, heavy damage) (see *Materials and Methods*).

[d] Key to designations for appearance of solution: M, milky; ST, semitranslucent; T, translucent).

mix 2 in the range of 0.5–1.0% APSA-80. To create mix 1, limonene was added to a solution of APSA-80, and best results (96.1% mortality) were obtained when APSA-80 was used at only 0.5%, which also resulted in the greatest number of large limonene droplets (see below). In mix 2, limonene was mixed with APSA-80 before being added to water, and efficacy increased with increasing amounts of APSA-80. For all mixtures, limonene solutions containing 1.5% APSA-80 caused high mortality (87.9–98.2%) but were usually phytotoxic (Table 1). All limonene solutions containing 0.5% APSA-80 were milky (opaque and white) (Table 1). Limonene solutions containing 1% APSA-80 were transparent if the limonene was first mixed with APSA-80 before being added to water (mixes 2 and 3). These same two mixes were semitransparent and grayish brown when they contained 1.25–1.5% APSA-80. Mortality associated with mix 6 (insects dipped in distilled water) averaged 1.3%; mix 7 (APSA-80 alone used at 0.50–1.50%) killed an average of 8–30% of mealybugs, with the highest mortality (30%) at 1.50% APSA-80; mix 8 (APSA-80 from 0.50 to 1.50% plus 0.1% Silwet) killed an average of 34.0–47.1%, achieving the maximum (47.1%) at 0.50% APSA-80 (data not shown for mixes 6–8).

Table 2. ANOVA for mealybug mortality (arcsine square-root transformed) associated with 1-min dips in 1% limonene solutions having various amounts of APSA-80 (0.5–1.5%) within each of five mixture types

	df	Mean square	F	$Pr > F$
Model	11	0.17	4.0	0.0002***
Rep	2	0.13	3.0	0.05*
APSA-80 (continuous variable)	1	0.12	2.8	0.13
Mixture type	4	0.20	4.5	0.003**
APSA-80*mixture	4	0.13	3.1	0.02*
Error	63	0.04		

Asterisk(s) indicate statistically significant terms in the model (type III sums of squares): *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Size of Emulsified Limonene Droplets. Mix 1, 2, and 3 solutions containing only 0.5% APSA-80 had a relatively large number of emulsified droplets of limonene regardless of mixing method (Fig. 1). These emulsified droplets made these solutions opaque and milky. Approximately 58% of the limonene droplets were large (>0.005 mm in diameter) when limonene was added directly to a 0.5% APSA-80 solution (mix 1), whereas only 10% were large if the limonene was first mixed with the APSA-80 concentrate before being added to water (mix 2) (Fig. 1). Solutions containing 0.75 or 1.0% APSA-80 were translucent or semitranslucent. These solutions contained relatively few visible limonene droplets, and almost all of the droplets were small. Solutions containing 1.25% and 1.5% APSA-80 were semitranslucent and contained particulates of unknown composition that were generally larger and more numerous than the emulsified droplets of limonene present within the same solutions. The particulates imparted a grayish brown coloration and made solutions look cloudy. Excluding from analysis those solutions containing 1.25 and 1.50% APSA-80, mix 2 solutions had significantly more small droplets and significantly fewer large droplets of limonene than mix 1 solutions (error df = 18, P = 0.05 for each comparison, Tukey's HSD). Across mix types, solutions containing 0.50% APSA-80 had a greater number of large and a greater number of small droplets than solutions containing 0.75 or 1.0% APSA-80; the solution containing 0.75% APSA-80 also had significantly more small droplets than the solution containing 1.00% APSA-80 (error df = 18, P = 0.05 for each comparison, Tukey's HSD).

Efficacy and Phytotoxicity of Best Limonene Mixture. The best limonene mixture (mix 3 containing 1% limonene, 0.75% APSA-80, and 0.1% Silwet) controlled from 69 to 100% of mealybugs and scales, depending on the species, insect stage, and application method (Table 3). At half strength, this mixture controlled 99–100% of whiteflies and aphids (Table 3). In side-by-side greenhouse tests, the 1% limonene mixture was equal to or superior to two standard nontoxic treatments typically recommended for control of scales and mealybugs. Mortality of green scales on potted gardenia plants averaged 95, 89, and 88% on plants sprayed with limonene, insecticidal soap, or horticultural oil, respectively, based on the number of

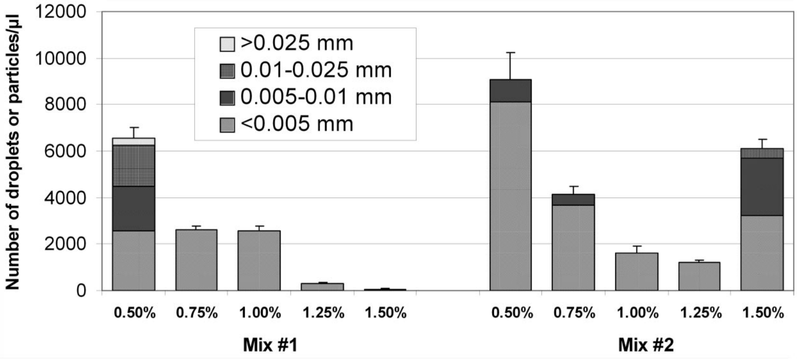

Fig. 1. Number and diameter of emulsified droplets and/or particles in solutions of 1% limonene containing different amounts of APSA-80 (0.50–1.50%) mixed by adding limonene to APSA-80 solution (mix 1) or to APSA-80 concentrate (mix 2). Height of error bars, 1 SEM (n = 4).

living green scales on treated and untreated (sprayed with water) plants counted 6 d after spraying (four replications of treatments, n = 2,580 living green scales in test) (Table 4). The limonene treatment also provided the highest level of control of third and fourth instars of mealybugs infesting potted gardenia, although percentage of control was relatively low because insects were tightly clustered in plant axils and thereby physically protected from sprays.

In phytotoxicity tests, about one-half of species tested showed no damage when dipped for 1 min in a 1% limonene solution. Plants not damaged included anthrurium; areca palm; bamboo palm; *Leucadendron;* ti, *Cordyline terminalis;* and sago palm. However, limonene dips damaged dracaena, laua'e fern, creeping club moss, monstera, and red ginger (Table 5). Symptoms included wilting, spots that were water-soaked or necrotic, loss of sheen, and brown leaf tips.

Table 3. Percentage of control of insect pests treated with limonene solutions

Pest	Host	Application method	Rate[a]	Stage	% dead	n[b]
Aphids						
Palm aphid, *Cerataphis brasiliensis* (Hempel)	Palm leaves	1-min dip	0.5×	Nym + Ad	99.8[c]***	464
Mealybugs						
Coconut mealybug, *Nipaecoccus nipae* (Maskell)	Coconut palm leaves	Spray	0.5×	Nymphs	95.2[d]*	207[e]
			0.5×	Adults	98.6[d]*	441[e]
			1.0×	Nymphs	100.0[d]*	207[e]
			1.0×	Adults	100.0[d]*	441[e]
Longtail mealybug	Green beans	1-min dip	1.0×	Third/fourth instars	92.0[c]*	284
Root mealybug	Gardenia roots	1-min dip	1.0×	Eggs	100.0[c]***	29
				Nymphs	95.3[c]***	66
Scales				Adults	93.0[c]**	161
Florida red scale, *Chrysomphalus aonidum* (L.)	Coconut palm leaves	Spray	0.5×	Eggs + Cra	91.1[d]*	361[e]
			0.5×	Nym + Ad	89.1[d]*	378[e]
			1.0×	Eggs + Cra	97.2[d]*	361[e]
			1.0×	Nym + Ad	97.6[d]*	378[e]
Green scale, *Coccus viridis* (Green) (test 1)	Gardenia leaves	1-min dip	0.5×	All instars	69.2[c]***	1,828
			1.0×		91.7[c]***	2101
Green scale (test 2)	Gardenia leaves	1-min dip	1.0×	All instars	87.9[c]***	823
		Spray	1.0×		68.9[c]***	1,147
Hemispherical scale	Orchid leaves	Spray	1.0×	Eggs	80.1[c]**	440
				Nym + Ad	98.6[c]***	76
Whiteflies						
Spiraling whitefly, *Aleurodicus dispersus* Russell	Plumeria leaves	Spray	0.5×	Eggs	100.0[c]*	40
				Nymphs	99.0[c]**	172
				Pupae	100.0[c]*	187
A. antidesmae	Gardenia leaves	Spray	0.25×	Nym + Pup	85.8[c]**	221

All tests used three to five replications of treatments. Treatment mortality was significantly higher than control mortality at *P = 0.05, **P = 0.01, or ***P = 0.001 (ANOVA or least significant difference means separation). Aqueous solution of 1% limonene, 0.75% APSA-80, and 0.1% Silwet L-77. Limonene and APSA-80 were first mixed as concentrates. Ad, adults; Cra, crawlers; Nym, nymphs; Pup, pupae.

[a] A 1× rate refers to the full-strength solution (undiluted with water) described above.

[b] Total number of insects treated.

[c] Percentage of mortality corrected for control mortality by Abbott (1925) formula.

[d] Percentage of killed estimated from counts of survivors in matched samples of treated and untreated material at the end of the test: % mortality = [(no. alive in control) − (no. alive in treatment) ÷ (no. alive in control)] * 100.

[e] Number of insects treated estimated as the number of living insects counted in matched set of control samples at the end of the test.

Table 4. Effect of spray treatments using 1% limonene, 2% soap, or 2% horticultural oil on scales and mealybugs infesting gardenia in the greenhouse

	No. surviving scales[a]	% mortality of mealybugs[b]
Water control	391.0a	3.7a[c]
1% Limonone[d]	18.5b	44.1c
2% Insecticidal soap[e]	43.3b	22.7bc
2% Horticultural oil[f]	45.8b	12.5ab

[a] *C. viridis*, all instars combined.
[b] *P. citri*, third and fourth instars.
[c] Means followed by the same letter(s) are not significantly different (Tukey's HSD, $\alpha = 0.05$).
[d] Aqueous solution of 1% limonene, 0.75% APSA-80 and 0.1% Silwet L-77. Limonene and APSA-80 were first mixed as concentrates.
[e] A 2% solution of Safer Insecticidal Soap (49% potassium salts of fatty acids).
[f] A 2% solution of SunSpray Ultra-Fine horticultural spray oil (98.8% paraffinic oil).

Discussion

Our results show that limonene is an effective natural alternative to mineral oils that can be used to wet and kill wax-covered insects such as mealybugs, scales, and whiteflies. Limonene is a naturally occurring monoterpene found in citrus, other fruits, conifers, and spices. Limonene is the major component in oil recovered from citrus rind when fruits are juiced (Florida Chemical 2004). It has a strong pleasant odor that is typically associated with the smell of oranges or lemons. Limonene is used in a variety of foods and beverages and is classified by the U.S. Food and Drug Administration as a Generally Recognized as Safe (GRAS) compound when used as a food additive or flavoring (EPA 1994). Limonene is frequently included as an ingredient in cleaning solutions, particularly those that are designed to cut grease or remove wax or oil (Florida Chemical 2004). The cost of technical grade d-limonene in truckload quantities generally varies from $0.60 to $1.00 per pound (Laurie Winget, personal communication).

Finding a suitable emulsifier for limonene and discovering how to mix it to achieve efficacy without phytotoxicity was a major obstacle during preliminary research. Limonene sometimes formed an unnoticeable surface slick in treatment solutions being using in dip bioassays. Under these conditions, control of mealybugs was generally close to 100%, but leaves dipped into solutions developed water-soaked spots that later became necrotic. Several limonene emulsifiers were tested, but these compounds created emulsions that were not very effective against mealybugs. The breakthrough came when APSA-80 was used, because at the lowest concentration necessary to remove the surface slick, there was not a dramatic reduction in efficacy as had occurred with other emulsifiers. Another advantage of this chemical is that it is registered with Environmental Protection Agency as an adjuvant for agricultural chemicals, permitting its use at any level in pesticide formulations. Solutions of APSA-80 up to 3% were tolerated by gardenia leaves in my research (unpublished data).

Limonene solutions containing 0.5% APSA-80 were milky (opaque and white) because a large amount of the limonene was present as a macroemulsion. In mix 1 (limonene added to APSA-80 solution), the presence of large droplets of limonene was not only associated with increased efficacy against mealybugs but also a higher level of phytotoxicity (mix 1, 0.5% APSA-80; Table 1). In this mix, higher percentages of APSA-80 (0.75 and 1.0%) were associated with a progressive decrease in efficacy, possibly because solutions contained both fewer and smaller visible drop-

Table 5. Phytotoxicity caused by limonene treatment solutions

Species	Limonene treatment				Symptoms[a]
	0.5%	1%	2%	4%	
Anthurium spathe, *Anthurium andraeanum* Linden	N[b]	N	L	M	Several necrotic spots on spathe
Anthurium foilage, *A. andraeanum*	N	N	S	L	Leaves with necrotic spots
Areca palm, *Chrysalidocarpus lutescens* (Bory) H. Wendl.	N	N	S	L	Slight loss of epicuticular wax (dull color), brown tips on leaves, water-soaked spots
Green dracaena, *Dracaena deremensis* Engl. 'Warneckii'	S	S	L	M	Some leaves showed necrotic spots
Laua'e fern, *Phymatosorus scolopendria* (Burm. f.) Pic.Serm.	S	S	L	M	Leaves wilted and some with necrotic spots
Leucadendron, *Leucadendron* 'Safari Sunset'	N	N	N	N	
Creeping club moss, *Lycopodium cernuum* L.	N	L	L	M	Tips of branches turned brown, some wilting
Monstera, *Monstera deliciosa* Liebm.	L	L	M	M	Leaves showed wilting and necrotic spots
Bamboo palm, *Rhapis excelsa* (Thunb.) Henry ex Rehd.	N	N	N	S	Leaves showed wilting and slight loss of color
Red ginger, *Alpina purpurate* (Vieill.) K. Schum.	N	S	L	M	Flower showed necrotic spots and general wilting
Red ti leaves, *Cordyline terminalis* (L.) Kunth	N	N	S	L	Leaves showed necrotic spots and wilting
Sago palm, *Cycas revoluta* Thunb.	N	N	N	L	Tips of some leaves turned brown

Plant material was dipped for 1 min in test solutions comprised of limonene, APSA-80, and Silwet L-77 at a ratio of 1.0, 0.75, and 0.10, respectively.
[a] Evaluations were made 2, 4, and 7 d after treatment.
[b] Cosmetic damage ratings: N, no damage; S, slight, generally unnoticeable, acceptable for sale; L, low level of damage, but would render commodity unsaleable; M, moderate damage (obvious from several meters distance); and H, high degree of cosmetic damage.

lets of limonene (Fig. 1). Emulsions made by adding an oil (such as limonene) to a solution of an emulsifier require more energy to give a good emulsion, and tend to break and separate more readily. This may partly explain the relatively large variability in mortality response associated with solutions made using mix 1. Efficacy trends were in the opposite direction for mix 2 solutions in the range of 0.5–1.0% APSA-80. Mix 2 solutions had the same composition as mix 1 solutions, but they were created by first mixing APSA-80 and limonene together as concentrates. The relatively low efficacy for mix 2, 0.5% APSA-80 solution is surprising, because this solution contained relatively many of the large limonene droplets (Fig. 1). I hypothesize that the reason for this opposite efficacy trend is related to chemical interactions involving APSA-80 and limonene that are more pronounced when these two ingredients are mixed as concentrates.

The addition of 0.1% Silwet L-77 to treatment solutions generally improved efficacy against mealybugs without dramatically increasing phytotoxicity. It was noted at the outset of our research that the addition of this silicone-based nonionic surfactant reduced the size of air bubbles surrounding test subjects dipped in treatment solutions, apparently due to a reduction in surface tension. This is consistent with observations on spider mites by Cowles et al. (2000). Solutions containing 1% APSA-80 were transparent if the limonene was first mixed with APSA-80 before being added to water (mixes 2 and 3). These same two mixes were usually opaque and grayish brown if they contained 1.25–1.5% APSA-80, due to a precipitate ($<$0.04 mm in diameter), possibly comprised of fatty acids derived from the APSA-80. In all mixes, phytotoxicity was generally increased when APSA-80 concentrations exceeded 1.0%. In the transparent solutions, the limonene was apparently present in the form of a microemulsion (micelles); macroemulsions formed (making the solution milky white) when limonene was added to the APSA-80 solution instead of being first mixed with APSA-80 concentrate.

As of 1994, limonene was registered as a pesticide active ingredient in 15 products: for use against ticks and fleas, as an insecticide spray, as an outdoor dog and cat repellent, as a fly repellent on tablecloths, as an insect repellent for use on humans, and as a mosquito larvicide (EPA 1994). If a new pesticide product containing limonene as the active ingredient were developed for control of mealybugs and scales, registration of the product with EPA would be required. However, limonene is exempt from the requirement of a tolerance in food when it is used as an inert ingredient (or "occasionally active") as a solvent or fragrance in pesticide formulations (Code of Federal Regulations 2003). At least one U.S. patent (No. 5,653,991, published in 1997 by Robert L. Rod; USPTO 1997) refers to using various oil-based formulations of d-limonene, with or without a water carrier, against plant pests such as whiteflies. To my knowledge, the only product currently on the market intended for control of plant pests using limonene as the active ingredient is Orange Guard for Ornamental Plants (Orange Guard, Inc., Carmel Valley, CA). This product, which was originally developed for control of household pests and ants, contains 5.8% d-limonene. The label recommends a 1:4–1:6 dilution of the product with water for use on plants (Orange Guard, Inc. 2005).

The toxicity and neurotoxic effects of monoterpenoids (including d-limonene) are discussed by Coats et al. (1991), and the suitability of limonene for control of insect pests has been reviewed by Ibrahim et al. (2001). Several reports mention using limonene for control of plant pests, including use on pine seedlings to reduce egg clusters of a notodontid moth (Tiberi et al. 1999), and the use 1–6% limonene solutions on carrot to repel a psyllid (Aaltonen et al. 2000, as cited in Ibrahim et al. 2001). Hummelbrunner and Isman (2001) used tobacco cutworms to test acute and sublethal effects of topically applied monoterpenoid essential oil compounds (including d-limonene). They found that mixtures of different monoterpenes produced a synergistic effect on mortality, and they developed a proprietary monoterpene mixture containing 0.9% active ingredient for use against foliar feeding pests.

Unlike soaps and oils, limonene evaporates quickly from leaves, leaving no residues that might cause a delayed phytotoxic response. Despite its advantages, phytotoxicity concerns will limit the use of limonene on certain types of plants or plant parts. Limonene was phytotoxic to strawberries when used at concentrations exceeding 3%, and cabbage and carrot seedlings were damaged when concentrations exceeded 9% (Ibrahim et al. 2001). In my study, 1% limonene damaged moss, ferns, ginger, certain types of dracaena, and delicate flowers. These developed water-soaked areas that later became necrotic. However, limonene solutions generally caused no damage to ornamentals with thick, waxy leaves, such as palms, cycads, and orchids.

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